PB84-213149

Dimethyltin Dichloride: Investigations into Its Gastrointestinal Absorption and Transplacental Transfer

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1983

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. REC
EPA-600/J-83-220		PB84-213149

4. TITLE AND SUBTITLE
Dimethyltin Dichloride: Investigations into its Gastrointestinal Absorption and Transplacental Transfer (Journal Version)

5. REPORT DATE
1983

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

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10. PROGRAM ELEMENT NO.
AB1C1A

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/11

15. SUPPLEMENTARY NOTES
Journal of Toxicology and Environmental Health, 12:89-98, 1983.

16. ABSTRACT

Dimethyltin dichloride (DMDC) is commonly used as a stabilizer in PVC pipe used for transport of potable water. Learning deficiencies have been observed postnatally in pups from DMDC-treated dams. Studies were conducted with female Sprague-Dawley rats to determine whether DMDC was absorbed by the dam and transferred across the placenta to fetal blood and brain tissue. This was accomplished in three phases: (1) a comparison of absorption of organic and inorganic tin from drinking water, (2) a comparison of prenatal and postnatal levels of tin in the pups in cross-fostering studies, and (3) a [^{14}C]dimethyltin dichloride tracer study to determine whether organic tin passed to the pup intact. Major findings include: (1) DMDC is absorbed in the gastrointestinal tract of the dam much more rapidly than Sn^{2+}; (2) the more rapid absorption of DMDC results in higher concentration of tin in fetal blood and brain; and (3) in fetuses that receive tin as DMDC, both tin and the methyl carbon are absorbed by the dam and transferred to the blood and brain of the fetuses.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
	Unclassified	11
Release to Public	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

CERI

EPA-600/J-83-220
JOURNAL ARTICLE

DIMETHYLTIN DICHLORIDE: INVESTIGATIONS INTO ITS GASTROINTESTINAL ABSORPTION AND TRANSPLACENTAL TRANSFER

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Dimethyltin dichloride (DMDC) is commonly used as a stabilizer in PVC pipe used for transport of potable water. Learning deficiencies have been observed postnatally in pups from DMDC-treated dams. Studies were conducted with female Sprague-Dawley rats to determine whether DMDC was absorbed by the dam and transferred across the placenta to fetal blood and brain tissue. This was accomplished in three phases: (1) a comparison of absorption of organic and inorganic tin from drinking water, (2) a comparison of prenatal and postnatal levels of tin in the pups in cross-fostering studies, and (3) a [^{14}C]dimethyltin dichloride tracer study to determine whether organic tin passed to the pup intact. Major findings include: (1) DMDC is absorbed in the gastrointestinal tract of the dam much more rapidly than Sn^{2+}; (2) the more rapid absorption of DMDC results in higher concentration of tin in fetal blood and brain; and (3) in fetuses that receive tin as DMDC, both tin and the methyl carbon are absorbed by the dam and transferred to the blood and brain of the fetuses.

INTRODUCTION

Organometal compounds have frequently been demonstrated to be more toxic than their corresponding metal ions. Dimethyltin is currently employed as a stabilizer in polyvinyl chloride (PVC) pipe used for transport of potable water supplies. Since dimethyltin leaches from PVC pipe into water, human exposure is possible (Guess and Stetson,

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We would like to express our appreciation to Dr. Judith Stober for her able assistance in statistical application. Also, the assistance of Patricia Underwood in the preparation of this manuscript is greatly appreciated.

This report has been subjected to the agency's peer and administrative review policy and approved. Approval signifies that the contents reflect the views and policies of the United States Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Journal of Toxicology and Environmental Health, 12:89-98, 1983
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1968). The ability of some aquatic plants and microorganisms to convert inorganic tin to alkyltin derivatives (Hodge et al., 1979; Hallas et al., 1982) may also contribute to increased alkyltin accumulation in water.

A variety of physiological effects of dimethyltin exposure have been documented (Penninks and Seimen, 1980; Thayer, 1974; Barnes and Stoner, 1959). Learning deficiencies have been observed in pups from dams exposed through their drinking water to dimethyltin dichloride (DMDC). In tests conducted previously in this laboratory, the behavior of organotin-treated animals differed significantly from control animals. Animals exposed to 40 mg DMDC/l exhibited decreased locomotor activity in a home cage situation (D. H. Taylor, 1982 personal communications). Pups from dams exposed to monomethyltin, dimethyltin, or trimethyltin exhibited deficient extinction learning ability in an appetitive learning paradigm and in a one-trial learning test (Noland et al., 1982).

The present work was undertaken to determine whether the organic tin was absorbed by the dam and crossed the placenta to pup blood and brain, and to determine when and how rapidly tin passes from dam to pup. This was accomplished in three phases:

1. a comparison of absorption of organic and inorganic tin,
2. a comparison of prenatal and postnatal levels of tin in the pups, and
3. a tracer study to determine if the organic tin passed to the pup intact.

METHODS

Seventy-day-old Sprague-Dawley (CD) strain rats were obtained from Charles Rivers breeding laboratory.

In Phase I the female animals were randomly assigned to one of three groups, two of which received the test compounds in their drinking water. Groups were exposed to 40 mg tin/l in solution as either dimethyltin dichloride ($n = 13$), while a second group received identical levels of tin as stannous chloride ($n = 13$). A third group received distilled water and served as a control group ($n = 12$). The exposure began 2 wk prior to breeding and continued through breeding and gestation. During this period, animals were housed one per cage with sawdust bedding in a room with constant temperature (22°C) and humidity (54%). Food (Purina Lab Chow) and water were available *ad libitum*. At birth and prior to first nursing, the pups were removed from the dam. The pups were sacrificed, blood samples were taken by carotid artery puncture, and brains were removed. All blood samples from a litter were pooled to make one sample of approximately 1 ml blood. Brains taken from the pups were also pooled to make one sample per litter. Dams were bled via abdominal aorta

puncture. Weight gain and water consumption were measured throughout the experiment.

In Phase II, a cross-fostering study was conducted. Except for the omission of the stannous chloride group, the animals were treated as described above. At birth, before first nursing, pups born to DMDC-exposed dams were placed either with control dams for rearing ($n = 8$) (DM/CT) or with another DMDC-exposed dam ($n = 8$) (DM/DM). Pups born to control dams were placed either with DMDC-exposed dams ($n = 8$) (CT/DM) or with another control dam ($n = 8$ (CT/CT). Brain and blood samples were collected from the remaining control ($n = 4$) and DMDC-exposed ($n = 4$) litters as described above. Subsequent brain and blood samples of the 4 groups were taken on postnatal d 10 and 21.

Since there is no available method specific for measurement of organotin in biological fluids, the tissues were analyzed for total tin content. The tissues were analyzed for tin by flameless atomic absorption technology. The determinations were performed on a Perkin-Elmer atomic absorption spectrophotometer model 305A, equipped with an electrodeless discharge lamp, model HGA2100 graphite furnace control, model HGA 74 graphite furnace, model AS1 autosampler, and a model 56 2-mV recorder. The samples were prepared for analysis by digestion with nitric acid and perchloric acid on a hotplate. To minimize interferences, the samples were treated with ammonium molybdate and then analyzed by the method of standard additions. The sensitivity and detection limit for the method are 0.005 μg/ml or 0.25 μg/sample and 0.004 μg/ml or 0.20 μg/sample, respectively.

In the tracer study (Phase III), 19-d pregnant dams were dosed by stomach tube with a 0.9 *M* NaCl solution containing 100 μCi DMDC/l (methyl-^{14}C, specific activity 6.9 mCi/m*M*, from New England Nuclear) at a dose of 0.8 ml/100 g body weight. Dams were sacrificed by decapitation at 5 min, 15 min, 30 min, 1 h, 2 h, 6 h, and 24 h ($n = 4$ at each interval) after dosing. Brains and 5-ml blood samples were taken for analysis. The fetuses were removed in order to take their blood and brain samples. Four samples from each litter were pooled to make one composite sample. Samples were prepared for counting via oxidation in a Packard Model B0306 sample oxidizer with 3 ml OXI-SCINT and 17 ml OXI-AMIN scintillation fluid. Samples were counted in a Beckman Model LS8100 scintillation counter. All samples were adjusted for quench and normalized.

Data for the first phase were analyzed using a Kruskal–Wallis test, and the means were compared with a non-parametric multiple comparisons procedure based on Kurskal–Wallis ranked sums (Hollander and Wolfe, 1973). Data for the cross-fostering phase were analyzed using an analysis of variance (ANOVA) (Hollander and Wolfe, 1973). Drinking-water consumption data were analyzed using a two-way nested ANOVA.

RESULTS

The Kruskal-Wallis test was used to test for differences in total tin content between controls and experimental groups in the first phase of experiments (Table 1). In the dams' blood there were significant differences among dosage groups ($X^2 = 23.8$, df = 2, $p < 0.001$). A nonparametric multiple comparison (based on Kruskal-Wallis ranked sums) showed that the tin content of blood from control dams and from dams given stannous chloride was not different, while the tin content from those given DMDC was significantly higher than both of the other groups. Analysis of pup blood and brain yielded similar results. The tin content in blood from pups of dams given DMDC was significantly higher than blood from pups of either of the other two groups ($X^2 = 24.7$, df = 2, $p < 0.0001$). The tin content from the brains of pups of dams administered DMDC was significantly higher than controls or those given $SnCl_2$ ($X^2 = 23.1$, df = 2, $p < 0.0001$). Dam body weights did not differ throughout the experiment. Dams exposed to DMDC drank significantly less than controls (Fig. 1) or those exposed to $SnCl_2$ ($f = 3.50$, df = 1.6, $p = 0.025$). As a result, the $SnCl_2$ animals consumed an average of 50% more tin than did those exposed to DMDC.

In the cross-fostering experiments, the level of tin in the blood of dams remained constant throughout the experiment. The levels in DMDC-exposed dams ($X \pm SE = 5.88 \pm 0.83$ μg/g) was significantly higher than that of controls ($X \pm SE = 0.115 \pm 0.04$; $p = 0.005$) (Fig. 2). The highest levels of tin in blood at any time in any age group were demonstrated at birth in gestationally exposed animals (Fig. 2). By 10 d postnatal age, blood tin levels decreased rapidly in both prenatally exposed groups. Pups exposed only postnatally (CT/DM) had blood levels of tin significantly lower than the DM/DM and the DM/CT animals. Both the CT/CT and CT/DM groups were found to be statistically lower than the

TABLE 1. Results of the Kruskal-Wallis Multiple Comparison for Tissue Tin Content

Dose (mg tin/l)	Dam blood		Pup blood		Pup brain	
	Mean (μg/g)	Group[a]	Mean (μg/g)	Group[a]	Mean (μg/g)	Group[a]
Dimethyltin dichloride (40 mg/l)	10.68	A	14.63	A	1.17	A
Stannous chloride (40 mg/l)	0.19	B	0.59	B	0.42	B
Distilled water control	0.14	B	0.20	B	0.32	B
Overall	$X^2 = 23.8$, df = 2, $p < 0.0001$		$X^2 = 24.7$, df = 2, $p < 0.0001$		$X^2 = 23.1$, df = 2, $p < 0.0001$	

[a]Means with the same group letter are not statistically different.

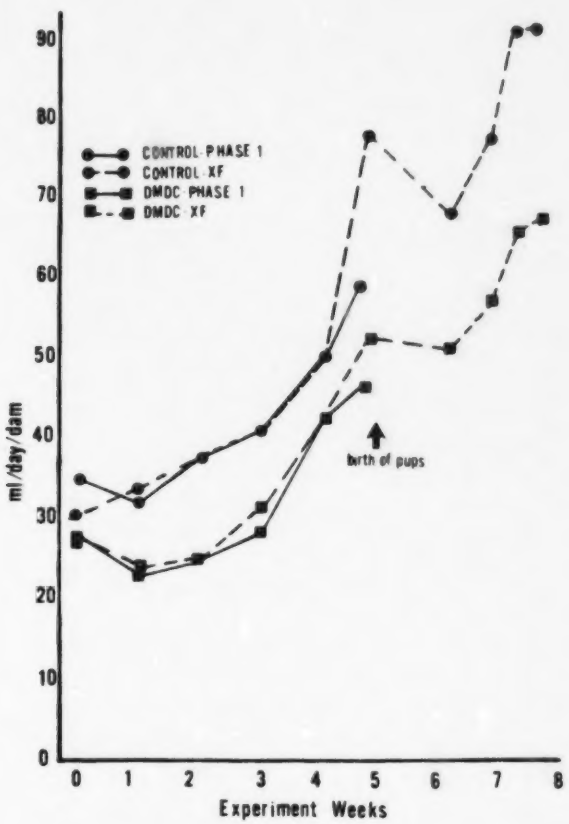

FIGURE 1. Dam water consumption throughout gestation and lactation was plotted as ml/d·dam against experiment week. Solid lines represents the phase 1 study, in which dams are sacrificed at parturition (wk 5); dotted lines represent the cross-fostering study (X-F), where water consumption measurements were continued through lactation (wk 8).

other 2 groups, but not different from each other ($f = 5.81$; df = 3,21; $p = 0.05$). At 21 d, all 4 groups were different from each other ($f = 27.33$; df = 3,12; $p = 0.01$).

Tin levels in the brains of prenatally exposed pups were again highest at birth and different from controls ($p = 0.004$) (Fig. 3). At 10 d, the DM/DM pups demonstrated significantly higher levels of tin in the brain than the other groups ($f = 19.42$; df = 3,12; $p = 0.01$). The DM/CT group was also different from the other groups. At 21 d, the DM/DM group was significantly higher than the CT/DM group, which was higher than either the CT/CT or DM/CT group ($f = 9.445$; df = 3,21; $p = 0.05$).

Results of the [^{14}C]DMDC study are presented graphically in Figs. 4

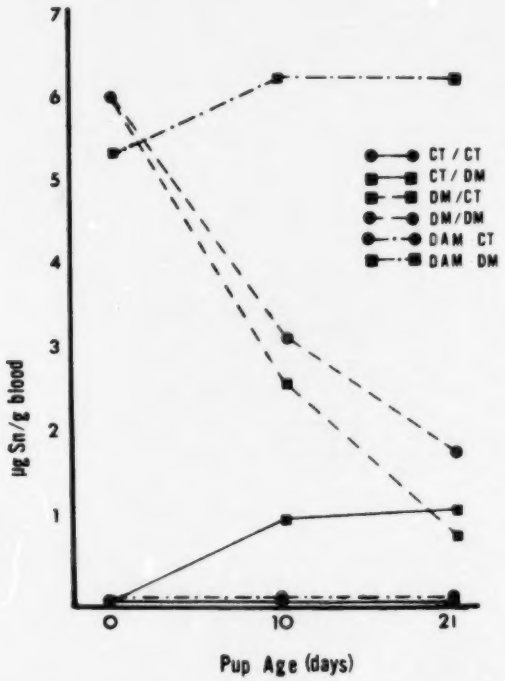

FIGURE 2. Tin found in cross-fostered pup blood, plotted as µg Sn/g blood, against pup age (d). At birth before first nursing, pups born to DMDC-exposed dams were placed with control dams for rearing (DM/CT) or placed with another DMDC-exposed dam (DM/DM). Pups born to control dams were placed with DMDC-exposed dams (CT/DM) or placed with another control dam (CT/CT). Each point represents $n = 4$. Note that pups were cross-fostered between dams at birth and that dam treatment did not change.

and 5. The highest measured level of label in the dam blood was recorded at 1 h, while the level in the brain continued to increase through 24 h when measurements were stopped. The label in the pup blood reached its highest measured level at 6 h, and in the brain the highest concentration measured was the last sample taken at 24 h after exposure. The level of DPM/g in dam brain at 24 h post dosing were equivalent to DPM/g in pup brain at 6 h post dose.

DISCUSSION

The results of this study have demonstrated that tin from DMDC is absorbed by the rat dam, undergoes transplacental transfer, and arrives in the brain of the prenatal animal. Inorganic tin, on the other hand, is poorly absorbed by the dam and is poorly transferred to the pups. From

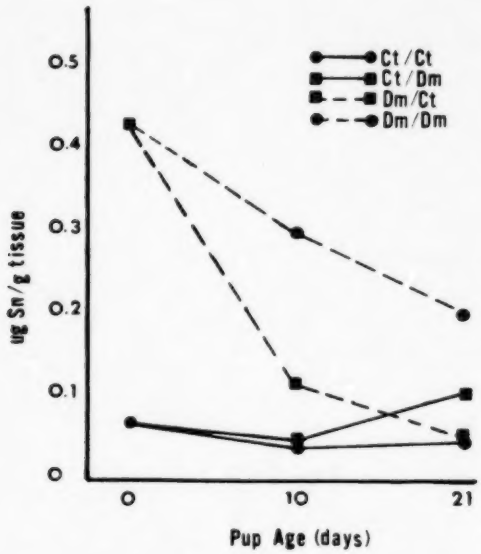

FIGURE 3. Tin found in cross-fostered pup brain, plotted as µg Sn/g tissue, against pup age (d). Legend as explained in Fig. 2. Each point represents $n = 4$.

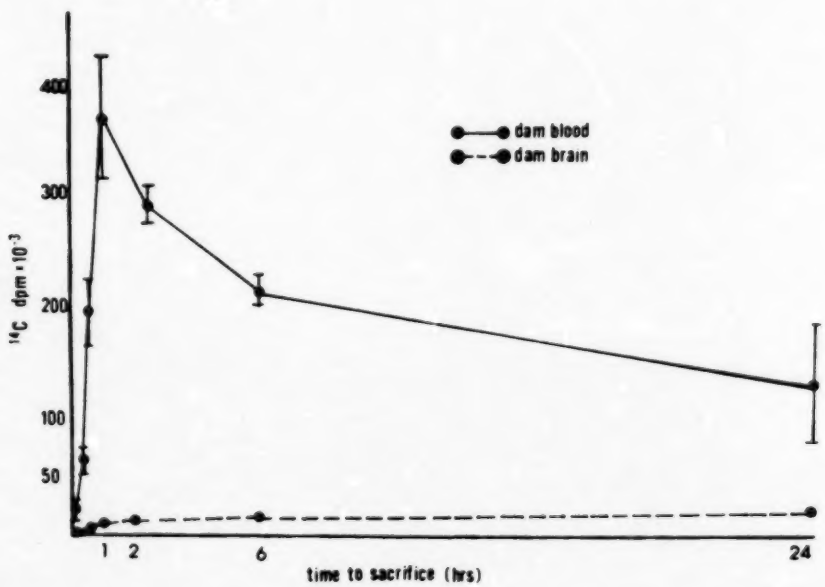

FIGURE 4. Dam blood and dam brain ^{14}C disintegrations/min found at time (in h) after intubation with dimethyltin dichloride (methyl-^{14}C). Measures are for g wet tissue weight or ml blood.

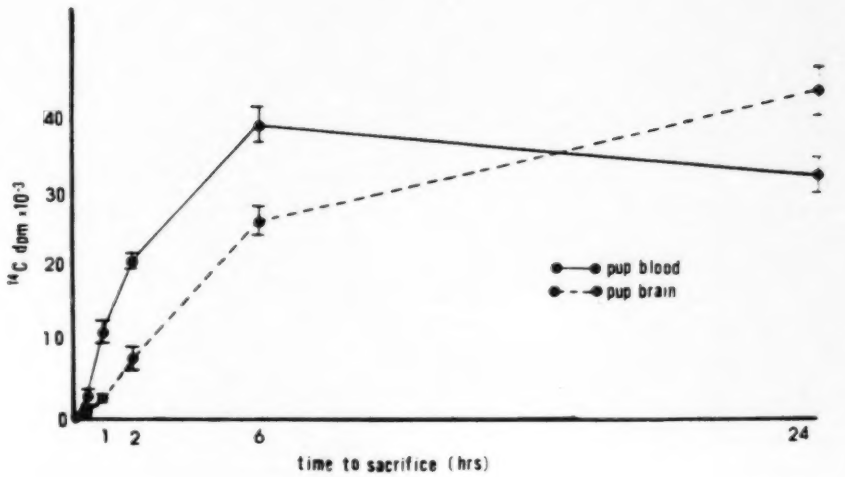

FIGURE 5. Pup blood and pup brain ^{14}C disintegrations/min found at time (h) after intubation with dimethyltin dichloride (methyl-^{14}C). Measures are for g wet tissue weight or ml blood. Each point represents $n = 4$.

this data it can be inferred that the methyl substitution on the tin greatly enhances the gastrointestinal absorption and subsequent transplacental transfer of tin.

The cross-fostering experiment demonstrates that the bulk of the tin is transferred from the pups prenatally. There is a 50% decrease in the amount of tin in the blood of the DM/DM pups 10 d after birth, and anothner 50% decrease from that level at 21 d, despite the increased water consumption of the dam. The lactationally exposed group never reached blood tin concentrations comparable to the levels at birth in pups exposed prenatally. Since dam blood tin levels do not change during gestation and lactation (Fig. 2), this indicates either a decreased amount of tin transferred in the milk or a decreased amount absorbed by the pups. The greatest decrease is seen in pups that received tin through gestation and then were transferred to control dams, indicating a rapid clearance of the tin from the blood and brain.

The level of tin in the brain of gestationally exposed pups was highest at birth. During the postnatal period, the tin from dimethyltin follows roughly the same pattern of decreasing concentration in pup brain and pup blood. This indicates relatively unimpeded brain-to-blood transfer, as is expected in animals with immature blood–brain barriers.

When DMDC (methyl-^{14}C) was administered by intubation, the label was readily absorbed into both dam blood and brain and fetal blood and brain. However, at 6 h post dosing, fetal blood ^{14}C levels are only 16% of

dam blood levels, yet fetal brain ^{14}C levels are 167% of dam brain ^{14}C concentrations. This 10-fold higher brain/blood ratio for fetal ^{14}C might be explained by either or both of the following mechanisms.

1. The "blood–brain barrier," which inhibits DMDC transfer into dam brain, has not yet developed in the 19-d-old fetus; consequently, DMDC crosses more rapidly from fetal blood to fetal brain than from dam blood to dam brain. If this is the explanation, one would expect that fetal brain and blood Sn levels would also demonstrate at least this high a brain/blood ratio during the longer exposures demonstrated in Figs. 2 and 3. However, the reverse is true. Pup blood Sn is 12–13 times higher than pup brain levels at partum. Consequently, this mechanism will not explain the entire effect seen in this tracer experiment.
2. [^{14}C]DMDC is metabolized in the dam or in the placenta to [^{14}C]non-Sn-containing metabolites. These metabolites of [^{14}C]DMDC cross the placenta and localize in fetal brain. These metabolites must be more available to fetal brain than DMDC- and/or Sn-containing metabolites. This would explain the higher fetal brain/blood ratios when ^{14}C is used as a tracer than when Sn is used to trace the movement of DMDC.

It is clear that inorganic Sn^{2+} will not be well absorbed across the GI tract (Table 1) and presumably does not readily cross the placenta and enter fetal brain. Consequently, the probability that non-Sn-containing [^{14}C]DMDC metabolites cross into pup brain is not exclusionary to the probability that the Sn-containing [^{14}C]metabolites and/or unchanged [^{14}C]DMDC also arrives in fetal brain.

This study shows (1) that DMDC is absorbed in the gastrointestinal tract of the dam much more rapidly than Sn^{2+}, and consequently that higher concentrations of tin are found in pup blood and brain from DMDC than from Sn^{2+} dosing, and (2) that the majority of the tin received as dimethyltin is transferred to the pups during gestation rather than lactation. DMDC dosing of dam results in both tin and methyl carbon absorption by the dam, transplacental transfer to fetal blood, and localization in fetal brain.

These data indicate that at least part of the increased central nervous system toxicity of DMDC over inorganic tin in drinking-water exposure could be the result of the increased availability of DMDC to the central nervous system of the dam and pup.

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Received March 1, 1983
Accepted March 11, 1983